

In the Claims:

Please amend the claims as set forth hereinafter.

Listing of Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1 - 14. (Canceled)

15. (Withdrawn) A method for identifying a MAR sequence using a Bioinformatic tool comprising computing values of one or more DNA sequence features corresponding to DNA bending, major groove depth and minor groove width potentials, and melting temperature.

16. (Withdrawn) The method of claim 15, wherein said Bioinformatic tool contains algorithms, adapted to use of profiles or weight-matrices, to compute values for one or more of said DNA sequence features corresponding to DNA bending, major groove depth and minor groove width potentials, and melting temperature.

17. (Withdrawn) The method of claim 16, wherein said profiles or weight-matrices are based on dinucleotide recognition.

18 - 22. (Canceled)

23. (Withdrawn) The method of claim 63 wherein the DNA binding protein is SATB1, NMP4, MEF2, S8, DLX1, FREAC7, BRN2, GATA 1/3, TATA, Bright, MSX, AP1, C/EBP, CREBP1, FOX, Freac7, HFH1, HNF3alpha, Nkx25, POU3F2, Pit1, TTF1, XFD1, AR, C/EBPgammma, Cdc5, FOXD3, HFH3, HNF3 beta, MRF2, Oct1, POU6F1, SRF, V\$MTATA_B, XFD2, Bach2, CDP CR3, Cdx2, FOXJ2, HFL, HP1, Myc, PBX, Pax3, TEF, VBP, XFD3, Brn2, COMP1, Evil, FOXP3, GATA4, HFN1, Lhx3, NKX3A, POU1F1, Pax6, TFIIB or Vmw65 or a combination of two or more of these transcription factors.

24. (Withdrawn) The method of claim 15, wherein values for identifying DNA bending comprise between 3 ° to 5 °.

25. (Canceled)

26. (Withdrawn) The method of claim 15, wherein values for the identification of the major groove depth comprise between 8.9 to 9.3 Å and values for the identification of minor groove width comprise between 5.2 to 5.8 Å.

27. (Canceled)

28. (Withdrawn) The method of claim 16, wherein the melting temperature is between 55 to 75 °C.

29 -33. (Canceled)

34. (Withdrawn) A method for identifying a MAR comprising providing at least one filter detecting clusters of DNA binding sites, wherein said filter detects said clusters using profiles or weight matrices.

35 - 41. (Canceled)

42. (Withdrawn- Currently Amended) A method for increasing protein production activity in a eukaryotic host cell comprising introducing into said host cell a purified and isolated DNA sequence comprising a first isolated matrix attachment region (MAR) nucleotide sequence, wherein the MAR nucleotide sequence is selected from the group consisting of:

- i. a sequence comprising a purified and isolated DNA sequence of claim 65;
- ii. one or more sequences of

SEQ ID Nos:1 to 27 or a fragment or variant thereof that has protein production increasing activity;

- iii. a synthetic MAR sequence comprising natural human MAR elements or fragments thereof assembled between linker sequences, and

a sequence complementary to any of i. to iii.

43. (Withdrawn) The method of claim 42, wherein said purified and isolated DNA sequence further comprises a promoter operably linked to a gene of interest.

44. (Withdrawn- Currently Amended) The method of claim 42 43, wherein said purified and isolated DNA sequence further comprises at least a second isolated matrix attachment region (MAR) nucleotide sequence which is a MAR nucleotide sequence comprising:

- i. a purified and isolated DNA sequence comprising: a.) at least one bent DNA element, b.) and at least one binding site for a DNA binding protein,
- ii. one or more sequences of SEQ ID Nos:1 to 27 or a fragment or variant thereof that has protein production increasing activity with sequence ID NO: 25, or
 - a sequence complementary to sequence ID NO: 25, or
 - a sequence having at least 90% identity with said SEQ ID NO: 25,
- iii. a purified and isolated cLysMAR element or fragment thereof,
- iv. a synthetic MAR sequence comprising natural MAR elements or fragments thereof assembled between linker sequences, and

a sequence complementary to any of i.to iv.

45. (Withdrawn) The method of claim 44, wherein said first and at least second MAR sequences are located at both the 5' and 3' ends of the sequence containing the promoter and the gene of interest.

46-47. (Canceled)

48. (Withdrawn- Currently Amended) A method for transfecting a eukaryotic host cell, said method comprising

- a) introducing into said eukaryotic host cell at least one purified DNA sequence comprising at least one DNA sequence of interest and/or at least one first purified and isolated DNA sequence comprising at least one bent DNA element comprising at least 10% of the dinucleotide TA and/or at least 12% of the dinucleotide AT on a stretch of 100 contiguous base pairs and at least one binding site for a DNA binding protein, wherein said purified and isolated DNA sequence has protein production increasing activity according to claim 65,

b) subjecting said transfected eukaryotic host cell to at least one additional transfection step with at least one second purified DNA sequence of interest and/or with at least one second purified and isolated DNA sequence comprising a MAR nucleotide sequence or other chromatin modifying elements, and

c) selecting said transfected eukaryotic host cell.

49. (Withdrawn) The method of claim 48, wherein said DNA sequence of interest is a gene of interest coding for a protein operably linked to a promoter.

50. (Canceled)

51. (Withdrawn- Currently Amended) The method of claim 48, wherein said second purified and isolated DNA sequence is a MAR nucleotide sequence ~~selected from the group consisting of:~~

- i. ~~a purified and isolated DNA sequence comprising: a.) at least one bent DNA element, b.) and at least one binding site for a DNA binding protein,~~
- ii. ~~one or more with sequence ID NO: 25, or~~
- ~~a sequence complementary to sequence ID NO: 25, or~~
- ~~a sequence having at least 90% identity with said SEQ ID NO: 25~~
- ~~sequences of SEQ ID Nos:1 to 27 or a fragment or variant thereof that has protein production increasing activity,~~
- iii. ~~a purified and isolated cLysMAR element or fragment thereof, and~~
- iv. ~~a synthetic MAR sequence comprising natural MAR elements or fragments thereof assembled between linker sequences, and~~
- ~~a sequence complementary to any of i to iv.~~

52-54. (Canceled)

55. (Withdrawn –Currently Amended) A method for transfecting a eukaryotic host cell, said method comprising co-transfected into said eukaryotic host cell at least one first purified and

isolated DNA sequence comprising at least one DNA sequence of interest, and a second purified and isolated DNA comprising at least one MAR-nucleotide selected from the group consisting of:

- i. a purified and isolated DNA sequence of claim 65,
- ii. one or more sequences of SEQ ID Nos 1 to 27 or a fragment or variant thereof that has protein production increasing activity,
- iii. a synthetic MAR sequence comprising natural human MAR elements and/or fragments assembled between linker sequences, and

a sequence complementary to any of i. to iii.

56.-61. (Canceled)

62. (Withdrawn) A computer readable medium comprising computer-executable instructions for performing the method for identifying a MAR sequence of claim 15.

63. (Withdrawn) The method of claim 15, wherein a feature corresponding to one or more binding sites for DNA binding proteins is identified.

64. (Withdrawn) The method of claim 63, wherein said DNA binding protein is a transcription factor.

65. (Currently Amended) A purified and isolated DNA sequence comprising:

- a) at least one bent DNA element comprising at least 33% of the dinucleotide TA and/or at least 33% of the dinucleotide AT on a stretch of 100 contiguous base pairs, ; and
- b) at least one binding site for a DNA binding protein,

wherein said purified and isolated DNA sequence is

a MAR nucleotide sequence with sequence ID NO: 25, or

a sequence complementary to sequence ID NO: 25, or
a sequence having at least 90% identity with said SEQ ID NO: 25
~~—has protein production increasing activity greater than that of chicken lysozyme MAR (eLysMAR).~~

66. (Cancelled)

67. (Previously Presented) The purified and isolated DNA sequence of claim 65, wherein said bent DNA element comprises at least five contiguous AT or TA dinucleotides.

68. (Previously Presented) The purified and isolated DNA sequence of claim 67, wherein said bent DNA element comprises at least 10 contiguous AT or TA dinucleotides.

69. (Withdrawn) The purified and isolated DNA sequence of claim 65 comprising a MAR nucleotide sequence, wherein the sequence is selected from the group consisting of at least one of SEQ ID Nos:1 to 27, a sequence complementary thereof, and a fragment or variant thereof that has protein production increasing activity.

70. (Previously Presented) The purified and isolated DNA sequence of claim 65, wherein said DNA binding protein is a transcription factor.

71. (Previously Presented) The purified and isolated DNA sequence of claim 70, wherein the transcription factor is a polyQpolyP domain protein.

72. (Currently Amended) The purified and isolated DNA sequence of claim 65, ~~comprising a sequence selected from the group consisting of at least one of SEQ ID NOs:24 to 27, a sequence complementary thereof, and~~
~~a fragment or variant thereof that has protein production increasing activity, wherein the purified and isolated DNA sequence has a melting temperature of between 55 and 75° and a DNA bending value of more than 4 radial degrees~~

and wherein said binding protein is a transcription factor.

73. (Cancelled)

74. (Previously Presented) A vector comprising a first purified and isolated DNA sequence according to claim 65 and a gene of interest.

75. (Previously Presented) The vector of claim 74, wherein said vector further comprises a second purified and isolated DNA sequence comprising at least one bent DNA element and at least one binding site for a DNA binding protein.

76. (Previously Presented) The vector of claim 75, wherein said purified and isolated DNA sequences are 5' and 3' to said gene of interest.

77. (Previously Presented) The vector of claim 74, wherein said vector further comprises one or more regulatory sequences.

78. (Previously Presented) The vector of claim 77, wherein said regulatory sequence comprises a promoter that is operably linked to said gene of interest.

79. (Previously presented) The vector of claim 77, wherein said regulatory sequence comprises an enhancer sequence.

80. (Currently Amended) The vector of claim 74, wherein said first purified and isolated DNA sequence is ~~selected from the group consisting of at least one of SEQ ID NOs:24 to 27, a sequence complementary thereof, and~~
~~a fragment or variant thereof that has protein production increasing activity, has~~ a melting temperature of between 55 and 75° and a DNA bending value of more than 4 radial degrees and wherein said binding protein is a transcription factor.

81. (Previously Presented) The vector of claim 75, wherein said second purified and isolated DNA sequence comprises at least 10% of the dinucleotide TA and/or at least 12% of the dinucleotide AT on a stretch of 100 contiguous base pairs.

82. (Currently Amended) The vector of claim 75, wherein said second purified and isolated DNA sequence is a MAR nucleotide sequence with sequence ID NO: 25, or
a sequence complementary to sequence ID NO: 25, or
a sequence having at least 90% identity with said SEQ ID NO: 25—selected from the group consisting of at least one of SEQ ID NOs:24 to 27, a sequence complementary thereof, and
a fragment or variant thereof that has protein production increasing activity, a melting temperature of between 55 and 75° and a DNA bending value of more than 4 radial degrees
and wherein said binding protein is a transcription factor.

83. (Previously Presented) The vector of claim 74, wherein said gene of interest is a structural gene.

84. (Previously Presented) The vector of claim 83, wherein said structural gene encodes an antibody or fragment thereof.

85. (Currently Amended) A transfected eukaryotic host cell comprising at least one purified-and isolated DNA sequence according to claim 65

comprising:

a) at least one bent DNA element comprising at least 33% of the dinucleotide TA and/or at least 33% of the dinucleotide AT on a stretch of 100 contiguous base pairs,

b) at least one binding site for a DNA binding protein,

wherein said purified DNA sequence is

a MAR nucleotide sequence with sequence ID NO: 25, or

a sequence complementary to sequence ID NO: 25, or

a sequence having at least 90% identity with said SEQ ID NO: 25.

86. (Previously Presented) The host cell of claim 85, further comprising at least one DNA sequence of interest.

87. (Previously Presented) The host cell of claim 86, wherein said at least one DNA sequence of interest comprises a structural gene.

88. (Currently Amended) The host cell of claim 87, wherein said at least one purified-and isolated DNA sequence and said at least one DNA sequence of interest are on the same vector.

89. (Currently Amended) The host cell of claim 87, wherein said at least one purified-and isolated DNA sequence and said at least one DNA sequence of interest are on separate vectors.

90. (Currently Amended) The host cell of claim 85, wherein said at least one purified-and isolated DNA sequence is selected from the group consisting of at least one of SEQ ID NOs:24 to 27, a sequence complementary thereof, and a fragment or variant thereof that has protein production increasing activity, has a melting temperature of between 55 and 75° and a DNA bending value of more than 4 radial degrees

and wherein said binding protein is a transcription factor.

91. (Previously Presented) A cell transfection mixture or kit comprising at least one purified and isolated DNA sequence according to claim 65.

92. (Withdrawn) A transgenic organism wherein at least some of its cells have stably incorporated therein at least one DNA sequence of claim 65.

93. (Withdrawn- Currently Amended) A method for increasing expression of a heterologous gene in a eukaryotic organism, said method comprising introducing into the said eukaryotic organism a purified and isolated DNA sequence according to claim 65.

94. (Cancelled)

95. (Cancelled)

96. (Cancelled)

97. (Withdrawn) The method of claim 93, wherein said purified and isolated DNA sequence is introduced on the same vector as said heterologous gene.

98. (Withdrawn) The method of claim 93, wherein said purified and isolated DNA sequence is introduced on a different vector from said heterologous gene.

99. (Withdrawn) The method of claim 93, wherein said eukaryotic organism is a mammal.

100. (Withdrawn) The method of claim 99, wherein said mammal is a human.

101. (Cancelled)

102. (Cancelled)

103. (Currently Amended) The vector of claim 74-101, wherein ~~said isolated and purified DNA sequence is selected from the group consisting of at least one of SEQ ID NOs:24 to 27, a sequence complementary thereof, and a fragment or variant thereof that has protein production increasing activity~~, wherein said bent DNA element comprises at least five contiguous AT or TA nucleotides and wherein said binding protein is a transcription factor and has a DNA bending value of more than 4 radial degrees.

104. (Cancelled)

105. (Currently Amended) A synthetic MAR sequence comprising natural human MAR elements and/or fragments thereof assembled between linker sequences, wherein the MAR sequence is sequence ID NO: 25, or

a sequence complementary thereto or sequence ID NO: 25, or
a sequence having at least 90% identity with said SEQ ID NO: 25 SEQ ID NOs: 24 to 27, and
a fragment or variant thereof that has protein production increasing activity, a melting
temperature of between 55 and 72° and a DNA bending value of more than 4 radial degrees,
wherein said bent DNA element comprises a transcription factor binding site.

106. (Currently Amended) The A synthetic MAR sequence comprising natural human MAR elements and/or fragments thereof assembled between linker sequences, wherein the human MAR sequence comprises a sequence is SEQ ID NO: 25, a sequence complementary thereto, and a fragment or variant thereof that has protein production increasing activity having a melting temperature of between 55 and 75° and a DNA bending value of more than 4 radial degrees, or
a sequence having at least 90% identity with said SEQ ID NO: 25,

wherein said sequence comprises at least 33% of dinucleotide TA and/or at least 33% of dinucleotide AT on a stretch of 100 continuous base pairs and a transcription factor binding site.

107. (Previously Presented) The synthetic MAR sequence of claim 106, wherein the linker sequences are BgIII-BamHI linkers.

108. (Withdrawn) A MAR sequence identified by the method of claim 15, wherein the MAR sequence has a protein production increasing activity greater than that of cLysMAR.

109. (Cancelled)

110. (Withdrawn) The method of claim 48, wherein said at least one additional transfection step is performed between 6 hours and 48 hours after the introduction of said first purified and isolated DNA sequence.

111. (Previously Presented) The purified and isolated DNA sequence of claim 71, wherein said transcription factor is selected from the group consisting of: SATB1, NMP4, MEF2, S8, DLX1, FREAC7, BRN2, GATA 1/3, TATA, Bright, MSX, AP1, C/EBP, CREBP1, FOX, Freac7, HGH1, HNF3alpha, Nkx25, POU3F2, Pit1, TTF1, XFD1, AR, C/EBPgamma, Cdc5, FOXD3, HFH3, HNF3 beta, MRF2, Oct1, POU6F1, SRF, V\$MTATA_B, XFD2, Bach2, CDP CR3, Cdx2, FOXJ2, HFL, HP1, Myc, PBX, Pax3, TEF, VBP, XFD3, Brn2, COMP1, Evil, FOXP3, GATA4, HFN1, Lhx3, NKX3A, POU1F1, Pax6, TFIIA or Vmw65 and a combination of two or more of said transcription factors.

112. (Currently Amended) A The purified and isolated DNA sequence of claim 65, comprising:

- a) ~~at least one bent DNA element comprising at least 33% of the dinucleotide TA and/or at least 33% of the dinucleotide AT on a stretch of 100 contiguous base pairs; and~~
- b) ~~at least one binding site for a DNA binding protein;~~

wherein said purified and isolated DNA sequence has protein production increasing activity.

113. (Previously Presented) The purified and isolated DNA sequence of claim 112, wherein said bent DNA element comprises at least five contiguous AT or TA dinucleotides.

114. (Previously Presented) A vector comprising a first purified and isolated DNA sequence according to claim 112 and a gene of interest.

115. (Previously Presented) The vector of claim 114, wherein said vector further comprises a second purified and isolated DNA sequence comprising at least one bent DNA element and at least one binding site for a DNA binding protein.

116. (Cancelled)

117. (Currently Amended) The vector of claim 74, wherein said first purified and isolated DNA sequence is SEQ ID No: 25, or a sequence complementary thereof, and a fragment or variant thereof that has protein production increasing activity having a melting temperature of between 55 and 75° and a DNA bending value of more than 4 radial degrees and wherein said binding protein is a transcription factor.

118. (Currently Amended) The vector of claim 82 74, wherein said vector further comprises a second purified and isolated DNA sequence has comprising at least one bent DNA element and at least one binding site for a DNA binding protein, wherein said second purified and isolated DNA sequence is selected from the group consisting of at least one of SEQ ID No: 25, a sequence complementary thereof, and a fragment or variant thereof that has protein production increasing activity having a melting temperature of between 55 and 75° and a DNA bending value of more than 4 radial degrees and wherein said binding protein is a transcription factor.

119. (Currently Amended) The host cell of claim 85, wherein said at least one purified and isolated DNA sequence is SEQ ID No: 25, a sequence complementary thereof, and a fragment or variant thereof that has protein production increasing activity, a melting temperature of between 55 and 75° and a DNA bending value of more than 4 radial degrees or a sequence having at least 90% identity with said SEQ ID NO: 25, and wherein said binding protein is a transcription factor.

120. (Cancelled)

121. (Cancelled)

122. (Currently Amended) The vector of claim 74 101, wherein said isolated and purified DNA has a DNA bending value of more than 4 radial degrees.

123. (Previously Presented) The purified and isolated DNA sequence of claim 72, having a melting temperature of between 55 and 62°.

124. (Currently Amended) A purified and isolated DNA sequence ~~having protein production increasing activity~~, wherein said DNA sequence comprises a) at least one bent DNA element, b) and at least one binding site for a DNA binding protein, and that it is a MAR nucleotide with SEQ ID NO: 25, or a sequence complementary thereof, or a sequence having at least 90% 70% identity with said sequence.

125. (New) The vector of claim 82, wherein said first DNA sequence and said second DNA sequence are located on a sequence distinct from the one containing a promoter and said gene of interest.

126. (New) The host cell according to claim 85, wherein the host cell is a high protein produced cell with a production rate of at least 10pg per cell per day.